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# *Small and ugly? Phylogenetic analyses of the "selfing syndrome" reveal complex evolutionary fates of monomorphic primrose flowers*

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## Abstract

One of the most common trends in plant evolution, loss of self-incompatibility and ensuing increases in selfing, is generally assumed to be associated with a suite of phenotypic changes, notably a reduction of floral size, termed the selfing syndrome. We investigate whether floral morphological traits indeed decrease in a deterministic fashion after losses of self-incompatibility, as traditionally expected, using a phylogeny of 124 primrose species containing nine independent transitions from heterostyly (heteromorphic incompatibility) to homostyly (monomorphic self-compatibility), a classic system for evolution of selfing. We find similar overall variability of homostylous and heterostylous species, except for diminished herkogamy in homostyles. Bayesian mixed models demonstrate differences between homostylous and heterostylous species in all traits, but net effects across species are small (except herkogamy) and directionality differs among traits. Strongly drift-like evolutionary trajectories of corolla tube length and corolla diameter inferred by Ornstein-Uhlenbeck models contrast with expected deterministic trajectories toward small floral size. Lineage-specific population genetic effects associated with evolution of selfing may explain that reductions of floral size represent one of several possible outcomes of floral evolution after loss of heterostyly in primroses. Contrary to the traditional paradigm, selfing syndromes may, but do not necessarily evolve in response to increased selfing.


## Introduction

The loss of self-incompatibility (i.e., post-pollination, pre-zygotic mechanisms that prevent self-fertilization; Igic et al. 2008) is widely acknowledged as one of the most frequent transitions in plant evolution (Stebbins 1950, 1970). Furthermore, it has important implications for microevolutionary processes (Igic et al. 2008) and macroevolutionary patterns of clade diversification (Takebayashi and Morell 2001; Goldberg et al. 2010; Ferrer and Good 2012; De Vos et al. submitted manuscript). Much of the evolutionary significance of the loss of self-incompatibility relates to the notion that it is necessary for the transition from allogamous (outcrossing) to predominantly

autogamous (selfing) mating (Stebbins 1970; Barrett 2002; Busch and Schoen 2008; Wright et al. 2008; Karron et al. 2012). While self-incompatible flowers are necessarily outcrossing, self-compatible flowers can either outcross, self or have an intermediate selfing rate, but high rather than low selfing is more common for self-compatible taxa (Raduski et al. 2012). Early works suggested that transitions toward selfing after the loss of self-incompatibility are associated with a suite of changes in morphological and reproductive floral characters (Darwin 1876, Ornduff 1969, Stebbins 1970), including decreased floral display, reduced pollen-to-ovule-number ratio, smaller distance between male and female organs within flowers (less herkogamy) and general reduction of floral size, collectively termed the "selfing syndrome" (see Table 1 in Ornduff 1969; Cruden 1977; Ritland and Ritland 1989; Goodwillie et al. 2010; Sicard and Lenhard 2011).

The selfing syndrome is considered a common phenomenon; transitions from outcrossing to selfing are thought to be "*in most cases*" (Sicard and Lenhard 2011, p. 1433) if not "*almost universally*" (Foxe et al. 2009, p. 5241) associated with the selfing syndrome. Stebbins (1970, p. 310) stated in an early discussion that "*in all self-fertilizers, flower size diminishes below that found in their cross-fertilizing ancestors*", suggesting that evolution toward a selfing syndrome upon the loss of self-incompatibility is a deterministic evolutionary trend. Most of our understanding of the evolution of floral traits in response to shifts toward increased selfing stems from analyses performed on a few taxa (e.g., in *Capsella*, Slotte et al. 2010; *Eichhornia*, Vallejo-Marín and Barrett 2009; *Leavenworthia*; Busch and Urban 2011; *Mimulus*, Ritland and Ritland 1989), or from informal interpretations of data on large numbers of species (e.g. Darwin 1876; Ornduff 1969; Stebbins 1970). Few comparative studies involving a larger number of species in an explicit phylogenetic framework have been conducted (but see Goodwillie et al. 2010 for an angiosperm-wide analysis of floral display in inflorescences and selfing rates). Specifically, although multiple, independent losses of self-incompatibility are documented in several clades (e.g., *Linanthus* section *Leptosiphon* (Polemoniaceae), Goodwillie 1999; Solanaceae, Goldberg et al. 2010; Triticeae (Poaceae), Escobar et al. 2010), it is unclear whether, or to what extent, replicate transitions in different species within a clade lead to similar patterns of phenotypic change (i.e., similar evolutionary trajectories). Are the floral displays of self-compatible species always smaller than those of their self-incompatible relatives, as Stebbins (1970) suggested? Do individual floral traits respond differently to the shift from outcrossing to selfing? Do different floral traits evolve synchronously or asynchronously with the loss

of self-incompatibility? These questions were identified as major gaps in our understanding of the transition to self-fertilization (Karron et al. 2012) and are addressed in the current study.

Several explanations that are not mutually exclusive have been proposed for the correlation between small flowers and selfing (Sicard and Lenhard 2011). First, small floral size may facilitate autonomous selfing and be directly targeted by selection, for instance, when selfing provides reproductive assurance under mate- or pollinator-limited conditions (Eckert et al. 2006). Second, if reproductive fitness is decoupled from the attractiveness of floral display for pollinators, as is the case in strict selfers, theory predicts that resources would not be invested in large flowers, but rather in increased reproduction (e.g. ovule production; Brunet 1992). Third, the selfing syndrome may be a pleiotropic effect of selection for small flowers driven by selection for the avoidance of herbivory (Eckert et al. 2006) or by selection for fast maturation in marginal habitats (Guerrant 1989; Aarssen 2000). These arguments suggest that after a transition toward selfing, floral size is under selection to progressively diminish in a range of scenarios.

Despite the broad acceptance of the selfing syndrome as a general phenomenon, the loss of self-compatibility does not necessarily result in small floral size. In fact, showy flowers with highly specialized pollination systems are often self-compatible and can have high selfing rates, in contrast with the prediction of the selfing syndrome (reviewed by Fenster and Martén-Rodríguez 2007). This conflict may be explained by the idea that showy, specialized flowers, relying on a small subset of the potential pollinator community, are inherently prone to reproductive failure, thus selfing may assure reproduction when outcrossing fails. This notion implies that self-compatibility and high selfing rates do not necessarily lead to small flowers, as predicted by the selfing syndrome. It is thus topical to ask whether floral traits respond to the loss of self-incompatibility consistently across different species.

In this study, we assess the extent to which the loss of self-incompatibility and the associated ability to self results in a deterministic trend of phenotypic evolution toward smaller floral size, as predicted by the selfing syndrome, using the primroses as our study system. This group of ca. 550 species (*Primula* and nested genera, Primulaceae, i.e. "/Primula" , where forward slash indicates clade name sensu Mast et al. 2001) is a classic model for the evolution of selfing, discussed in the seminal works of Ornduff (1969) and Stebbins (1970) as a prototypical example of repeated losses of self-incompatibility and associated origins of selfing, in the form of transitions from heterostyly to homostyly. Heterostyly is a form of heteromorphic incompatibility in which populations consist of two

(distyly) or three (tristyly) genetic morphs that differ in the reciprocal placement of sexual organs and in mating type, so that only crosses between morphs are fully fertile (reviewed by Ernst 1962; Ganders 1979; Barrett 1992; Wedderburn and Richards 1992; Barrett and Shore 2008; Cohen 2010; Naiki 2012). Homostylous species have only one floral morph (i.e., are monomorphic), and crossing experiments established that they are self-compatible, hence, self-fertilization is possible (Ernst 1962; Richards 2003). Detailed phylogenetic studies concluded that the crown node of /Primula was heterostylous and indicated several, deeply nested losses of heterostyly within the clade (Mast et al. 2006, De Vos et al. submitted manuscript). Similar patterns occur in many of the ca. 28 families that include heterostylous taxa, with homostylous species evolving multiple times independently from heterostylous ancestors (e.g. in *Amsinckia*, Boraginaceae, Schoen et al. 1997; *Narcissus*, Amaryllidaceae, Graham and Barrett 2004; *Nymphoides*, Menyanthaceae, Tipperey and Les 2011; Pontederiaceae, Kohn et al. 1996; *Turnera*, Turneraceae, Truyens et al. 2005). The recurrent transition from heterostyly to homostyly is an important model for floral evolution and the evolution of selfing in angiosperms (Barrett 2003), making it an ideal system to evaluate the selfing syndrome from a quantitative, comparative perspective.

Here, we analyze a large data set of continuous floral traits of /Primula by using a combination of recently developed comparative methods that employ explicit models of quantitative trait evolution and account for both evolutionary relationships and intraspecific variation. We assess whether floral evolution among heterostylous and homostylous lineages is congruent with predictions of a selfing syndrome, by asking: Do heterostylous and homostylous species differ in (i) overall floral size and (ii) individual floral traits? (iii) Is the pattern of phenotypic change (i.e. evolutionary trajectory) of each floral trait affected by the loss of heterostyly? By answering these questions, our study contributes to an improved understanding of the phenotypic consequences of the loss of self-incompatibility, one of the most important transitions in flowering plant evolution.

## Methods

### Phylogeny

In this study, we used the 265-taxon, time-calibrated phylogeny of Primulaceae (subfamily Primuloideae sensu Angiosperm Phylogeny Group 2009) estimated by De Vos et al. (submitted; Fig. S1 in Supplementary Information; TreeBASE reference TB2:S14824). Taxon sampling was designed to cover the morphological variation in the family, by including species from all genera and all sections, representing ca. 35% of extant diversity. Phylogenetic relationships were inferred by De Vos et al. (submitted) based on four chloroplast markers using the uncorrelated lognormal relaxed clock method in BEAST v.1.6.2 (Drummond and Rambaut 2007). Here, analyses were executed using a sample from the posterior distribution of trees of De Vos et al. after pruning all species outside of /Primula from each sample, or on a maximum clade credibility (MCC) tree with median node heights calculated from the pruned posterior distribution. Subsequently, we removed branches from the MCC tree and posterior distribution of trees representing species for which no quantitative floral data was available (see below).

### Morphological data

Quantitative floral measurements were assembled from three sources. First, detailed data on the floral morphology of *Primula* species were meticulously reported in the series "*Stammesgeschichtliche Untersuchungen zum Heterostyly-Problem*" by Ernst (1938, 1949, 1953, 1956, 1959, 1961, 1962) for a total of approximately 835 pages. These data, consisting of ten measurements on each of 2680 flowers representing 138 currently accepted species, were digitized using Optical Character Recognition software (Readiris Pro v.11, I.R.I.S. Group S.A., Louvain-la-Neuve, Belgium) on high-resolution scans, manually corrected, and proof-read twice. We followed the most recent comprehensive monograph of *Primula* for species synonymy (Richards 2003). Secondly, we extracted ranges and means of the respective floral traits from "Flora of China" (Hu and Kelso 1996) for the Chinese *Primula* species. The ranges listed in this treatment typically stem from observations on multiple herbarium sheets per species in multiple herbaria (pers. comm., S. Kelso), ensuring that intraspecific variation is adequately captured. Finally, for the species of *Dionysia*, we used the information provided in the monograph of Grey-Wilson (1989). We did not include measurements from species of the nested

genus *Dodecatheon*: their floral structure differs in at least six qualitative aspects from *Primula*-like flowers (discussed by Mast et al. 2004), impeding meaningful quantitative comparisons of floral organs to homologous traits of other species in the clade.

Among the available floral measurements, we selected four floral traits thought to influence reproductive function: the distance from the base of the flower to (a) the apex of the calyx (calyx length) and (b) the mouth of the corolla-tube (tube length), (c) the diameter of the corolla limb (corolla diameter), and (d) the absolute distance between the top of the male (anthers) and female (stigmas) organs within flowers (herkogamy). We included the compound trait herkogamy rather than the position of anthers and stigmas separately, because it is problematic to compare anther and stigma positions of species with and without heterostyly. Moreover, herkogamy has been shown to affect the genetic selfing rate (e.g. Herlihy and Eckert 2007) and the probability of autonomous self-fertilization (De Vos et al. 2012) and is therefore more meaningful to compare between heterostylous and homostylous species than the absolute position of sexual organs. Some floral characters for which data was available, for example, the length of the calyx teeth or the degree of incision of the corolla lobes, were excluded from further analyses, because we expected strong correlations with the traits we included. We provide boxplots of raw data in Fig. S2. Data available from the Dryad Digital Repository: doi:10.5061/dryad.vq2m6.

For analyses based on species means, we first scored a species' predominant breeding system (heterostyly or homostyly), relying on monographic (Ernst 1962; Grey-Wilson 1989; Richards 2003) and flora treatments (Hu and Kelso 1996). For instance, species with both heterostylous and homostylous populations may be heterostylous in most of their range, but homostylous in peripheral or isolated populations (e.g. *P. prolifera*) – such species were considered predominantly heterostylous. Subsequently, we calculated the means of the four traits listed above in all the 124 species of the phylogeny by De Vos et al. (submitted) for which data were available; other analyses incorporated intraspecific variation in breeding system and quantitative floral measurements.

**Transition rates and ancestral state inference**

To assess the number of independent losses of heterostyly captured by the taxon sampling we used a two-step approach in a likelihood framework. First, we estimated transition rates between heterostyly and homostyly, using three transition models. Model 1 is based on the genetic

underpinnings of heterostyly. The heterostyly S-locus "supergene" provides non-phylogenetic evidence that heterostyly is rarely gained but frequently lost in *Primula*. The supergene consists of at least three tightly linked (Mendelian) loci that regulate male and female aspects of heterostyly, as documented in classic crossing experiments Ernst (1920s-1950s; Lewis and Jones 1992). The evolution of homostyly is understood as the product of genetic recombination at the S-locus, hence, occurring instantaneously and frequently. Because, origin of heterostyly requires assembly of multiple genes at the S-locus and the elements of the S-locus have been consistently documented among greatly divergent *Primula* species, heterostyly is thought to have evolved once in *Primula* and lost repeatedly. Model 1 thus fixes the gain rate of heterostyly, $q_{01} = 0$, setting the root of /Primula at heterostyly. However, some phylogenetic studies suggested heterostyly may sometimes evolve many times in parallel among closely related taxa (e.g. six times in *Lithospermum*; Cohen and Davis 2012). Although for *Primula* heterostyly as ancestral state was demonstrated (Mast et al. 2006; De Vos et al. submitted), it is (at least theoretically) possible that heterostyly re-evolved within Primula after being lost. Therefore, we also include models where $q_{10}$ and $q_{01}$ are estimated from the data, either based on the 124 taxa for which we have quantitative data (model 2), or based on the full 265 taxa Primulaceae dataset of De Vos et al. (submitted; Fig. S1; model 3). All transition models accounted for the effects of heterostyly and homostyly on speciation and extinction rates documented by De Vos et al. (submitted) by employing BiSSE transition models (Maddison et al. 2007) implemented in the R package diversitree (FitzJohn 2012). We accounted for phylogenetic uncertainty by obtaining the rates for the MCC tree and for 100 trees from the posterior distribution. Direct statistical comparison of the three models is problematic because they are partly based on different data. Instead, we perform analyses on all models and assess congruence among results.

In the second step, we used estimated transition rates to infer the likelihood of presence/absence of heterostyly at ancestral nodes in the /Primula MCC tree (using asr.marginal in diversitree). We also generated stochastic character maps (Huelsenbeck et al. 2003) of 100 possible histories of the transitions between heterostyly and homostyly on the MCC tree and 10 on each tree of the posterior distribution, using make.simmap in the R package phytools (Revell 2012).

**Floral differentiation between heterostylous and homostylous taxa**

To quantify the extent of phylogenetic signal in all four traits, we used Pagel's (1999) lambda, a

scaling parameter of the off-diagonal elements of the phylogenetic variance-covariance matrix, as implemented in the phylosig function in phytools, because this measure performed comparatively well among a set of estimators of phylogenetic signal (Münkemüller et al. 2012). We determined if lambda was significantly different from zero using likelihood-ratio tests for each of 100 trees from the posterior distribution.

To summarize quantitative variation and covariation of all floral traits and to visualize trait disparity among species of either breeding system, we performed a phylogenetic principal component analysis. We used the function phyl.pca in phytools on mean values per species and trait using the MCC tree, employing the appropriate scaling factor for branch lengths determined as for the test for phylogenetic signal.

To test whether individual floral traits differ between species with and without heterostyly, we used four generalized linear mixed models, one for each floral trait, implemented in the R package MCMCglmm (Hadfield 2010), which accounts for both intraspecific variation and phylogenetic relatedness of species. We used “presence of heterostyly” as predictor variable, fitted a univariate normal response to the data of each floral trait, and included phylogeny and intraspecific variation as random variables. Models were run for 50,000 iterations after a burn-in of 5,000 iterations and a thinning interval of 50 iterations on each of 100 trees from the posterior distribution. Post-burnin samples generated on each tree were then combined. We adjusted the standard, weak priors to facilitate convergence by splitting the observed total variance in our response variables in equal parts between the random (phylogenetic and intraspecific) and the residual variance components, and we ensured that effective samples sizes were sufficiently high (>10,000). We assessed the predictor's significance by determining if the 95% credible interval (95% CI) of the effect size included zero.

**Models of trait evolution**

To test whether the evolutionary trajectories of floral traits differ between heterostylous and homostylous species, we fitted a series of likelihood models for continuous characters and compared the estimated parameters among supported candidate models. To this end, we modeled quantitative trait evolution as a Ornstein–Uhlenbeck (OU) stochastic process, which describes a combination of random drift (termed Brownian Motion; BM) and a deterministic, selective "pull" toward an optimal value, termed $\theta$ (Hansen 1997; Butler and King, 2004; Beaulieu et al. 2012). The evolution of the trait

toward $\theta$ is governed by a constant describing the strength of selection, termed $\alpha$, and a constant that measures the intensity of drift-like random fluctuations in the evolutionary process, termed $\sigma^2$. When $\alpha$=0, the model collapses to BM (hereafter $BM_1$); when $\alpha$>0, the model is termed $OU_1$, where subscript "1" refers to the presence of a single, global optimum $\theta$ (i.e. expected value). Although these models employ terms similar to those used for microevolutionary processes (e.g. drift, selection), they actually describe the pattern of phenotypic change , hence require careful interpretation (Beaulieu et al. 2012; Hansen & Martins 1996). To clarify, the second central moment of the OU-process (its variance, $\sigma^2$) is proportional to additive genetic variance and inversely proportional to effective population size, which both affect the rate of random genetic drift (Lande 1976). Since additive genetic variance may differ among species (e.g. due to their mating system, Lande 1977) and it may correlate with effective population size (as models often assume), the relation between genetic drift and $\sigma^2$ may differ among species. In addition, under a pure neutral model of genetic drift with mutation, the asymptotic rate at which allopatric populations diverge is determined by the mutational variance, but not by effective population size (Lynch & Hill 1986). Thus, $\sigma^2$ is related to, but does not equate with, random genetic drift. To avoid confusion, we make an explicit distinction between genetic drift, which is a population-genetic process, and macroevolutionary drift, described by the model parameter $\sigma^2$, throughout the paper.

Recently, OU models have been generalized to incorporate multiple values for $\theta$, $\alpha$, and/or $\sigma^2$ that can be associated with transitions of discrete character-states along the phylogeny (Butler and King 2004, O'Meara et al. 2006, Beaulieu et al. 2012). The mapped history of a character (here, presence or absence of heterostyly) divides the phylogeny in heterostylous and homostylous partitions; $\theta$, $\alpha$, and/or $\sigma^2$ are then fitted to the quantitative data with global or partition-specific values. By comparing support for models that either have single or multiple values for $\theta$, $\alpha$, and/or $\sigma^2$, we can thus determine which aspects of the evolutionary trajectory change with the loss of heterostyly (Table 1).

We considered five models with multiple values of $\theta$, $\alpha$ and/or $\sigma^2$. The $BM_S$ model includes one global, optimal (expected) trait value $\theta$, but the intensity of the stochastic fluctuations, $\sigma^2$, can differ along the phylogeny as determined by the presence or absence of heterostyly (O'Meara et al., 2006). The $OU_M$ model, with two values of $\theta$ but one $\alpha$ and one $\sigma^2$, describes the situation where a floral trait may evolve toward different optimal values, for instance indicated by a smaller $\theta$ for homostylous than heterostylous species, while the rate of evolution toward these optima is the same (Butler and King

2004). Beaulieu et al. (2012) recently implemented expanded $OU_M$-models in which, besides $\theta$, also $\alpha$ or $\sigma^2$ vary with the character history ($OU_{MA}$ and $OU_{MV}$, respectively). In the most general case, $\theta$, $\alpha$ and $\sigma^2$ are each estimated separately for heterostylous and homostylous tree partitions ($OU_{MVA}$; Beaulieu et al. 2012).

Implementation

To compare how well these seven models (two models with global and five models with multiple values for $\theta$, $\alpha$ and/or $\sigma^2$, respectively) fit the floral-trait data of heterostylous and homostylous species, we assigned each species to either breeding system and used the 100 stochastic maps generated for the MCC tree under each of the three transition models described above. We chose to use stochastic maps, rather than the maximum likelihood estimate, to allow for incorporation of uncertainty in the evolutionary history of heterostyly in the estimation of differences between the evolutionary trajectories of floral traits of heterostylous and homostylous species, a strategy that was found to be useful in other studies (e.g. Price et al. 2013). We confirmed that results were robust against phylogenetic uncertainty, by perfoming analyses on 100 stochastic maps generated across 10 trees from the posterior distribution, including the two maximally different trees (based on Robinson-Foulds symmetric distance). It was computationally unfeasible to run analyses across a larger sample of trees.

Models were fitted using the R package OUwie (Beaulieu et al. 2012). To facilitate model fitting, we divided all trait values by ten and adjusted the initial values of the likelihood search, trying values of 0.01, 0.3, or 1.0. Nevertheless, for most mapped histories, it was impossible to fit the most complex model, $OU_{MVA}$, to all trait data and $OU_{MA}$ to corolla diameter data. Inspection of the eigendecomposition of the Hessian matrix and examination of the eigenvectors revealed that problematic inference was usually related to difficulties in estimating $\alpha$ jointly with $\sigma^2$ from the data. Therefore, the aforementioned models were considered too complex for the information contained in the data and abandoned (Beaulieu et al. 2012). We also excluded mapped histories for which the maximum likelihood could not be determined reliably in all models as indicated by negative eigenvalues of the Hessian (Beaulieu et al. 2012). Model fit was determined using AICc weights calculated from $\Delta$AICc scores (Burnham and Anderson 2002). AICc weights can be interpreted as the probability that a model is the best one among candidate models. We considered parameterizations of models with AICc weight < 0.05 to be statistically not supported by the data. We interpreted results by



 

comparing differences in parameter estimates between heterostylous and homostylous species, jointly considering estimates under all supported models for each trait, as advised by Beaulieu et al. (2012).

Expectations

The selfing syndrome theory predicts that flowers should become smaller in homostylous than heterostylous species. Therefore, models with two optima, $\theta$ ($OU_M$, $OU_{MA}$, $OU_{MV}$) are expected to receive higher AICc weights than models with one ($BM_1$, $BM_S$, $OU_1$) and the inferred $\theta$ should be smaller for homostylous species. Secondly, although not traditionally considered part of the syndrome, selfing may purge genetic variation and increase homozygosity, leading to decreased effective population sizes, which in turn implies that genetic drift may become more important in the evolutionary process in homostylous compared to heterostylous species (Lloyd, 1980; Hamrick and Godt, 1996). We expect higher AICc weight for the model that allows for two $\sigma^2$ ($OU_{MV}$), with higher $\sigma^2$ for homostylous species. Finally, the self-compatible flowers of homostylous species rely less on providing an adequate fit to their pollinator(s) for reproduction than obligately outcrossing, heterostylous species. Consequently, homostylous species are expected to be less affected by selective constraints imposed on floral traits by pollinators than heterostylous species. Instead, homostylous species are expected to experience stronger selection for low herkogamy to facilitate self-fertilization. Therefore, we predict that the model with two $\alpha$ ($OU_{MA}$) receives high AICc weight for all traits, with homostylous species having higher $\alpha$ for herkogamy, but lower $\alpha$ for other traits, compared to heterostylous species.

# Results

### Transition rates and ancestral state inference

The inferred rate of transition from heterostyly to homostyly was similar under models 1-3 (mean±SE expected transitions per lineage per MY across all stochastic maps 0.094±0.002, 0.094±0.003, and 0.060±0.001, respectively). The gain rates of heterostyly differed considerably (model 1, fixed at 0; model 2, 0.911±0.028; model 3, 0.005±0.0001). These rate differences were reflected in the number of transitions mapped among the 124 taxa: model 1, fixed at 1 gain, 14.1±0.01

losses (mean±SE across all stochastic maps); model 2, 47.8±0.54 gains, 48.1±0.51 losses; model 3, 1.8±0.04 gains, 13.3±0.05 losses. Figure 1 illustrates the ancestral states at internal nodes of the MCC tree as the proportion of likelihood associated with presence/absence of heterostyly under each transition model. While heterostyly was ancestral for the /Primula clade under model 1 and 3, with at least nine losses nearly exclusively occurring on terminal branches, under model 2 the history of heterostyly is associated with large uncertainties. Hence, our phylogenetic sampling captures at least 9-13 independent losses of heterostyly, indicating that our dataset provides a good model to study the effects of the repeated loss of self-incompatibility on floral traits.

**Floral differentiation between heterostylous and homostylous taxa**

All quantitative floral traits showed significant phylogenetic signal (all traits $p<0.001$ on all trees, except corolla diameter: $p<0.05$ on 78% of trees, $0.05\leq p\leq 0.08$ on 22% of trees). Significant lambdas across the trees ranged 0.783-0.899 (calyx length), 0.703-0.807 (tube length), 0.339-0.631 (corolla diameter), and 0.705-0.845 (herkogamy). This justifies analysis and interpretation of the floral data in a phylogenetic context.

The phylogenetic principal component analysis produced four axes (PCs) that explained 50.6%, 20.9%, 15.5% and 13.0% of variance, respectively. PC1 was negatively correlated with all traits (factor loadings indicated in Fig. 2; values ranging -0.58 and -0.77), whereas PC2 was correlated strongly and positively with herkogamy (factor loading 0.75) and negatively with corolla diameter (factor loading -0.46). The scatterplot diagram of PCs 1 and 2 showed that PCA scores of species with and without heterostyly largely overlapped on axis 1, but species without heterostyly had generally lower scores on PC 2 (Fig. 2).

The MCMCglmm analyses, which accounted for intraspecific variation and phylogenetic relatedness of species, indicated that all investigated floral traits were significantly different between homostylous and heterostylous species (Fig. 3). 95% credible intervals (CIs) of posterior means of heterostylous and homostylous species overlapped considerably (Fig. 4), but CIs of effect sizes of homostyly did not include zero for any trait (Fig. 3). Directionality of change differed among traits, as signs of effect sizes differed among traits. In contrast with expectations under the selfing syndrome, homostylous species tended to have slightly longer corolla tubes and calyces than related heterostylous species. Congruent with the selfing syndrome, corolla diameter and herkogamy tended to be smaller in

homostylous species.

**Models of trait evolution**

The results of model-fitting for six models of quantitative trait evolution are summarized in Table 2 as means across 100 stochastic maps with standard error and associated AICc weights. Here, we report results based on transition model 3 using the posterior distribution of trees, because its transition rates are inferred from most data; results under all models and on MCC trees were qualitatively identical and quantitatively highly similar (Table S1).

The best model for calyx length was $OU_1$ (AICc weight 0.50), where the optimum $\theta$ was equal between homostylous and heterostylous species. $OU_M$, $OU_{MA}$, and $OU_{MV}$ also received considerable AICc weight (0.28, 0.11, and 0.11 respectively), and yielded equal estimates of $\theta$ of 0.53 for homostyles and 0.69 for heterostyles, congruent with our predictions, while estimates of $\sigma^2$ and $\alpha$ did not differ with breeding system.

The best model for corolla tube length was $OU_{MV}$ (AICc weights 0.52). $\theta$ was slighly smaller for homostylous than heterostylous species (1.29 and 1.51, respectively) and $\sigma^2$ was 3.3-fold higher in homostyles (1.19 versus 0.36 in heterostyles), which fits our prediction that the macro-evolurionary trajectory of change is more drift-like in homostylous than in heterostylous species. The $OU_{MA}$ model received less support (AICc weight 0.43) and indicated a slightly higher $\alpha$ for homostylous than heterostylous species (0.63 vs. 0.59). This would be contrary to our expectations.

The diameter of the corolla limb was best modeled under either $OU_1$ or $OU_{MV}$ (AICc weights 0.43 and 0.41, respectively). Homostylous and heterostylous species had similar $\theta$ (1.20 and 1.27, respectively under $OU_{MV}$ and 1.27 for both under $OU_1$), but, congruent with the results of corolla tube length, $\sigma^2$ was again higher in homostylous species (7.73 versus 3.81 in heterostylous species). $OU_M$ also received some support (AICc weight 0.16), with parameter estimates similar to the $OU_1$ model.

For herkogamy, the $OU_{MV}$ model received with 0.92 nearly all AICc support. As expected, homostylous species had lower $\theta$ than heterostylous species (0.07 and 0.53, respectively). In contrast to the other traits, $\sigma^2$ was lower in homostylous species (0.23 versus 0.04 in homostylous species).

## Discussion

### Patterns of trait variability among species

The commonly expected effect of the loss of self-incompatibility on the evolution of floral traits is a deterministic trend toward small floral size and shifted resource allocation (i.e., the selfing syndrome), mainly because species that self have smaller returns from investment in traits that attract pollinators (Sicard and Lenhard 2011). Our results are partially congruent with evolution toward smaller floral traits after the loss of self-incompatibility (here, loss of heterostyly), as self-compatible species (i.e. homostyles) have slightly smaller selective optima, $\theta$, for all traits in most evolutionary models inferred to be plausible (Table 2). However, our analyses also suggest a more complex, versatile evolutionary fate of self-compatible lineages. First, we find that homostylous species span a similar range of variation in overall floral morphology as heterostylous species, with the exception of herkogamy (compare PC1 and PC2 and their factor loadings, Fig. 2). Second, our results suggest contrasting effects of loss of heterostyly among traits. While both Bayesian glmm and evolutionary-model fitting analyses indicate that all floral traits differ between heterostylous and homostylous species (Fig 3; Tables 2, S1), the glmm indicated that directionality of change differed among traits. Posterior means of corolla tube and calyx length were higher in homostyles, whereas corolla diameter and herkogamy were larger in heterostyles (Fig. 3). Third, optimal models for corolla diameter and corolla-tube length are characterized by 2 - 4.6-fold higher macroevolutionary drift, $\sigma^2$, in homostyles (Tables 2, S1). In contrast, differences between heterostyly and homostyly in optimal trait value, $\theta$, and the strength of the selective pull toward it, $\alpha$, were only slight (Tables 2, S1).

To put it simply: while some homostylous species appear to evolve toward a typical selfing syndrome after the transition to homostyly, others do not. Morphological patterns among some species illustrate overall decreased floral size of homostyles compared to heterostyles, as expected (compare e.g. homostylous *P. cicutariifolia* vs. heterostylous *P. merilliana; P. prenantha* vs. *P. serratifolia; P. muscoides* vs. putatively closely related *P. reptans*; Figs 1, S2; Chen 2009). Among other species, morphological patterns are strikingly different, incongruent with a selfing syndrome. For example, homostylous *P. halleri*, *P. scandinavica* and *P. scotica* likely evolved from a heterostylous, *P. farinosa*-like ancestor (Guggisberg et al. 2006, 2009), but *P. halleri* is much larger than the other species. Similarly, corolla diameter of homostylous *P. japonica* is largest among species in this study,

while closely related homostylous *P. prenantha* is among the smallest (Figs 1, S2). Taken together, the variability in magnitude and direction of change in floral traits among homostylous species detected by our analyses contrast with the traditional paradigm of the selfing syndrome and Stebbins' (1970) influential remark that selfing lineages *always* have smaller flowers than their outcrossing relatives.

The transition from heterostyly to homostyly is a classic system to investigate the genetic, ecological, and population biological contexts for the evolution of selfing (reviewed e.g. by Ernst 1955; Lewis and Jones 1992; Barrett 1992; Barrett 2003; Barrett and Shore 2008). Field experiments revealed a high capacity for self-fertilization in several homostylous primroses (e.g. Washitani et al. 1994; Carlson et al. 2008; Chen 2009; De Vos et al. 2012), although genetic estimates of the actual selfing rate are rarely available (Piper et al. 1984). Our analyses indicate that homostylous species have strongly reduced herkogamy (Figs 3, 4) and a lower selective optimum, $\theta$, for herkogamy (Table 2). Since herkogamy typically correlates negatively with the degree of auto-fertility (*Primula*, De Vos et al. 2012; *Aquilegia*, Herlihy and Eckert 2007) the low average herkogamy of many homostylous species suggests that they generally have selfing rates distinctly higher than self-incompatible, heterostylous species (Barrett and Shore 1987; De Vos et al. 2012). The transition from heterostyly to homostyly and the associated loss of self-incompatibility is thus a well-suited system for testing floral differentiation between outcrossing and (largely or partially) selfing species. We also note that it is possible that the ability of (some) homostylous species to reproduce both autogamously and allogamously plays a role in explaining the high variability of their floral traits, in comparison to their obligately outcrossing, heterostylous relatives. For instance, extent of divergence in floral size between homostylous species and closely related heterostylous species might relate to the amount of herkogamy in homostylous species (Fig. S3). Finally, homostyly is sometimes associated with polyploidy (Richards 2003), but there is no strict correspondence (Naiki 2012). Therefore, it is unlikely that effects of homostyly are confounded by effects of polyploidy.

We consider it to be unlikely that our results may be the product of methodological artifact, but we note theoretical limitations in current analytical methods. First, MCMCglmm and OUwie assume that groups of homostylous and heterostylous species each evolve under one evolutionary regime that is homogeneous among its species. However, large disparity of floral sizes among homostyles (Figs 2, S2) challenges the assumption that all homostylous species are exposed to exactly the same evolutionary forces (and the same is true, *mutatis mutandis*, for heterostyles). Moreover, methods

assume that evolutionary regimes operated invariably since the transition to homostyly, which need not be the case. For instance, evolution of the amount of herkogamy in homostyles may affect their potential for self-fertilization (discussed above), possibly altering selective constraints stemming from dependence on pollinators (see also Fig. S3). Thus, except effects due to heterostyly and homostyly, possible heterogeneity in evolutionary forces among species are not directly accounted for in our analyses.

Second, the fraction of species in /Primula that have homostyly is similar among the ca. 550 extant species (0.18; De Vos et al., submitted) and the 124 sampled species (0.15), hence our sampling reflects the known frequency of homostyly. However, because of the relative rarity of homostyly and its "tippy" distribution within the phylogeny (Fig. 1), tree partitions (sums of all branches mapped to be either heterostylous or homostylous) are of greatly unequal size. This poses the methodological challenge that differential strength of selection (i.e. multiple $\alpha$) among tree partitions becomes difficult to detect (Beaulieu et al. 2012). In addition, it is intrinsically challenging to distinguish effects of increased macroevolutionary drift, $\sigma^2$, from decreased selective constraints, $\alpha$. Both scenarios can explain increased average divergence between sister taxa, making high $\sigma^2$ and low $\alpha$ qualitatively two sides of the same phenotypic coin. Technically, their distinction relates to the shape of the decay curve of phylogenetic covariance with increasing branchlengths across the phylogeny (Hansen & Martins 1996), which is likely problematic to estimate from empirical data given uncertainty in measurement, phylogenetic relationships and character history. Problematic inference is reflected by lack of convergence for models with multiple $\alpha$ and multiple $\sigma^2$ ($OU_{MVA}$) and by $\sigma^2$ values under $OU_{MV}$ (e.g. corolla-tube length: high in homostyles, low in heterostyles) not always being mirrored in reciprocal differences in $\alpha$ under $OU_{MA}$ (low in homostyles, high in heterostyles; Tables 2, S1). Nevertheless, very similar sets of models were supported when accounting for phylogenetic uncertainty and under each transition model (Table S1), even though transition models affected probabilities of heterostyly and homostyly at deeper nodes, and in consequence the size of tree partitions (Fig. 1). These methodological considerations indicate that our results hold despite the challenge of co-esimtating $\sigma^2$ and $\alpha$.

Third, higher values of $\sigma^2$ for corolla-tube length and corolla-limb in homsotylous species (Table 2) illustrate that strong morphological divergence in homostylous species can arise over relatively short branches that imply a transition in breeding system (e.g. corolla diameter in *P.*

*bellidifolia* vs. *P. flaccida*; tube length in *P. halleri* vs. *P. farinosa*; Fig. 1). Such rapid evolution may also drive the seemingly contradictory results for calyx and corolla tube length, where OUwie estimated $\theta$ to be lower and the posterior means in MCMCglmm analyses to be higher in homostyles than in heterostyles (Table 2, Fig. 4). This contradiction can be explained by noting that posterior means in MCMCglmm may be strongly affected by aforementioned cases of rapid evolution, while in OUwie models, estimates of $\theta$ can remain relatively unaffected, because such rapid changes are accommodated through high $\sigma^2$.

Despite these methodological challenges and limitations, our results enable us to conclude that homostylous species display considerable phenotypic variation in floral traits – more than predicted by the deterministic paradigm of loss of self-incompatibility being associated with reduction of floral size. Instead, higher levels of stochasticity, $\sigma^2$, associated with loss of heterostyly (Table 2) suggest that evolutionary trajectories are likely to include values that are more extreme in homostyles than in heterostyles. For instance, species with floral tubes that are considerably longer than those of closely related species are usually homostylous (e.g. *P. halleri* in section *Aleuritia*; Richards 2003), and corolla tubes of homostylous species are both among the longest (*P. verticillata*) and shortest (*P. muscoides, P. cicutariifolia*) of all species (Figs 1, S2). Moreover, multiple, self-compatible lineages within /Primula are morphologically so distinct that they are frequently recognized as separate genera (i.e. *Dodecatheon, Cortusa, Sredinskaya*), whereas this is the case for only one self-incompatible lineage (i.e. *Dionysia*; Scott 1865, Richards 2003, Mast et al. 2004, Reveal 2009), reinforcing the notion that the loss of heterostyly does not necessarily limit the scope for morphological diversification in a deterministic trajectory toward small floral size.

**Possible causes of trait variability**

Which biological processes are affected by transition to homostyly and may explain our comparative results on effects of loss of self-compatibility? Homostylous species may experience relaxed selective constraints and increased developmental instability compared to heterostylous species. The increased selfing ability of homostylous species implies that they rely less on pollinators for successful reproduction. Therefore, pollinators should exert less stabilizing selection on the floral traits of homostylous species compared to heterostylous species (Cresswell 1998), an expectation congruent with the empirical finding that levels of floral integration may decrease after self-

incompatibility is lost (Anderson and Busch 2006, but see Rosas-Guerrero et al. 2011). Moreover, shifts to selfing may also affect the canalization of development, generating "random" phenotypic change at a high rate (Levin 1970; Vallejo-Marín and Barrett 2009). Developmental instability is commonly associated with high levels of inbreeding, especially in marginal populations or after hybridization (Levin 1970) – conditions proposed to promote the evolution of homostyly (Guggisberg et al. 2009). These processes could contribute to the relatively wide variation of floral traits among homostylous species (Figs 1, 2, S2).

Homostylous species may also have reduced effective population sizes. Sustained selfing over prolonged periods of time is expected to decrease effective population size, especially when selfing lineages experience recurrent episodes of small population size (Lloyd 1980; Charlesworth 2009). Such bottlenecks are more likely to occur in homostylous lineages, because their selfing ability may facilitate bottlenecks associated with establishment of new populations after dispersal over pre-existing geographic barriers (Baker 1955). The rate of random genetic drift correlates negatively with effective population size and may thus be more important in homostylous than heterostylous taxa (Lloyd 1980). The combination of increased genetic drift and release of selective constraints (discussed above) in homostyles suggest that changes in floral traits involved in pollinator attraction (e.g., corolla diameter) and interaction (e.g., corolla-tube length) are likely to become more easily fixed by neutral processes in homostylous than in heterostylous species, congruent with our finding of strongly drift-like trajectories for these traits (Tables 2, S1). In contrast, the importance of neutral process for calyx length may change relatively little upon the shift to homostyly (Table 2), as the calyx' primary function – protection of other floral organs during bud development – is not differentiated between homostylous and heterostylous species. Herkogamy is functionally more constraint in homostylous species, because it affects their selfing ability, unlike in heterostyles, explaining lower macroevolutionary drift in herkogamy of homostyles (Tables 2, S1).

Theoretical models predict that a selfing syndrome should evolve at a high rate when the loss of self-incompatibility is associated with directional selection toward new fitness optima (Glémin & Ronfort 2013), as in the well-known cases of *Capsella rubella* and *Leavenworthia alabamica* (Foxe et al. 2009; Guo et al. 2009; Busch et al. 2011; Sicard et al. 2011; Slotte et al. 2012). This is unlikely to be generally true in /Primula, because differences between heterostylous and homostylous species in posterior means (Figs 3, 4) and optima ($\theta$; Tables 2, S1) are small and trajectories of floral evolution

are not indicative of strong shifts in selection (Tables 2, S1). Instead, if the primary population genetic effect of the loss of self-incompatibility is not shifting directional selection but relaxing selective constraints (making phenotypic evolution more drift-like), then selfing syndromes should evolve rarely and much slower (Glémin and Ronfort 2013), a prediction more congruent with our results.

In addition, after transitions to homostyly, standing genetic variation is expected to gradually decline (Hamrick & Godt 1996), making trait evolution via sorting of standing genetic variation increasingly unlikely (Glémin & Ronfort 2013). However, when trait evolution occurs, it may be faster in homostyles compared to heterostyles, mainly because fixation time of beneficial alleles is shorter in selfers. Indeed, theoretical models predict that selfing slows down trait evolution only if effective population sizes are strongly reduced, for instance when homostyly is associated with increased incidence of population bottlenecks (discussed above; Glémin & Ronfort 2013).

In summary, during and after transition to homostyly, trajectories of floral-trait evolution may be jointly affected by the nature of selective constraints on floral form, rates of genetic drift, amount of standing genetic variation, and extent of alterations of effective population sizes.

**Conclusion**

The probability of selfing syndromes to evolve in primroses appears to be dependent on the nature of changes in several population genetic processes upon the evolution of homostyly and during floral evolution thereafter. The specifics of such changes may differ among homostylous lineages. For instance, bottleneck effects for population size may be more common in lineages where establishment of new populations after long-distance dispersal is likely, which in part depends on the degree of local landscape heterogeneity (Haller et al. 2013). Thus, population genetic changes and contingencies underlying the trajectory of evolution of floral traits may differ for independent origins of homostyly, possibly affecting outcomes of phenotypic evolution, even when the genetic mechanism through which homostyly originates would be identical among lineages. Thus, a reduction of floral size represents one of several possible outcomes of floral evolution after loss of self-incompatibility; selfing syndromes do not always evolve in response to a switch to increased autogamy. Multiple possible outcomes are reflected in drift-like macroevolutionary trajectories among homostylous primroses, in congruence with Ernst's (1962, p.94) characterization of heterostylous and homostylous flowers as "*an overall picture of surprising diversity of floral plasticity*" (translated from German by JMdV). It would be



 

useful to determine whether other aspects of the selfing syndrome, such as pollen/ovule ratios, display a similar variability among homostylous species (Ornduff 1969; Cruden 1977; Ritland and Ritland 1989). Investigating why the loss of self-incompatibility is associated with a selfing syndrome in some, but not all, lineages requires determining whether it reflects stochastic outcomes of neutral processes, or directional selective regimes diametrically different among species or varying over time. Compelling evidence for either scenario will probably stem from a combination of comparative methods and targeted experiments on reproductive ecology.

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**DATA ARCHIVING**

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**Figure 1.** Maximum clade-credibility chronogram and character states of the clade /Primula. Main figure: pie charts at internal nodes indicate the proportion of likelihood associated with the ancestral state being heterostylous (in red) or homostylous (in grey), based on transition model 3, in which rates of losses and gains of heterostyly were informed by a Primulaceae-wide analyses (Fig. S1). Bars to the right of the tree are drawn with length proportional to the mean value in mm per species of the four analyzed floral traits (left to right: calyx length, corolla-tube length, corolla-limb diameter, and herkogamy), where red and grey bars are used for heterostylous and homostylous species, respectively. The column Observations indicates the number of observations that were available to calculate species means and account for intraspecific variation in the MCMCglmm analyses. Inset: phylogenies with tip states indicated and pie charts indicating ancestral states as for the main figure, but based on model 1 (right inset figure), in which only losses of heterostyly are allowed, and model 2 (left inset figure), in which transistion rates are based only on the depicted tip states. Despite the apparent difference between the ancestral state reconstructions, the results of downstream analyses were qualitatively identical.

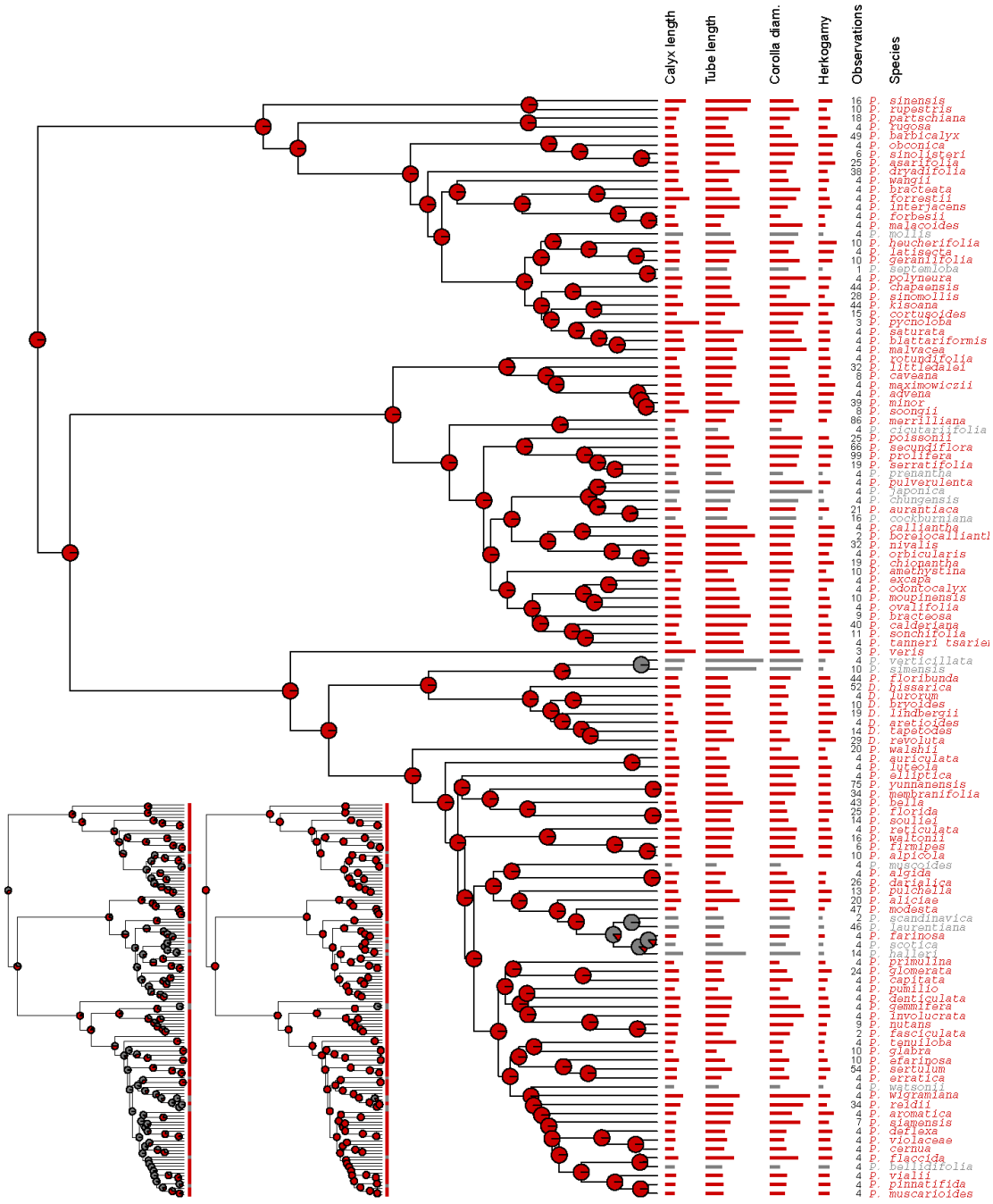

**Figure 2**. Scatterplot diagram of phylogenetic principal component analysis on four floral traits (calyx length, corolla-tube length, corolla-limb diameter, and herkogamy) for the first two principal components (PC1, PC2). Triangles and circles represent homostylous and heterostylous species, respectively. Arrows indicate factor loadings on PC1 and PC2.

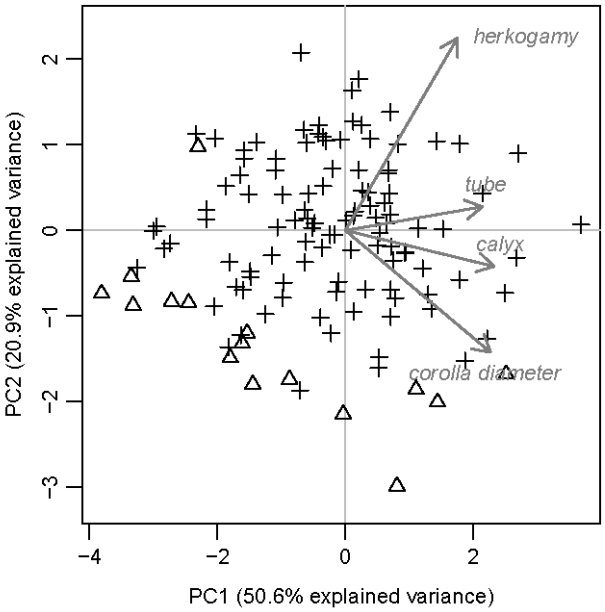

**Figure 3.** Posterior estimates of the effect size of homostyly on calyx length, corolla-tube length, corolla-limb diameter and herkogamy. Dots represent the mean of the posterior estimate with 95% credible intervals (CIs) represented by horizontal bars. Because the 95% CIs do not overlap with zero, all traits significantly differ between heterostylous and homostylous species: calyx and corolla tube are longer in homostylous species (95% CIs of the effect size 0.12 to 1.10 mm and 0.61 to 1.06 mm, respectively), whereas corolla diameter and herkogamy are larger in heterostylous species (95% CIs of the effect size -1.77 to -0.34 mm and -3.99 to -4.52 mm, respectively).

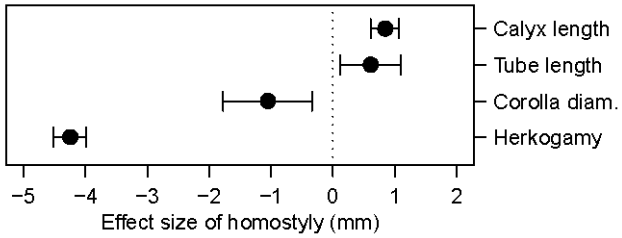

**Figure 4.** Posterior means of traits of heterostylous (Het.) and homostylous (Hom.) species in (A) calyx length (Het. 7.37 mm, Hom. 8.21 mm), (B) corolla-tube length (Het. 16.37 mm, Hom. 16.98 mm), (C) corolla-limb diameter (Het. 12.91 mm, Hom. 11.86 mm), and (D) herkogamy (Het. 5.44 mm, Hom. 1.19 mm), as inferred from the MCMCglmm analyses. Dots represent the overall meta-analytical posterior means, 95% credible intervals (CIs) are represented by the vertical bars. Although the overall posterior means overlap strongly between heterostylous and homostylous species, the effect due to homostyly is significant for all traits (because posterior estimates of effect sizes of homostyly do not include zero; see Fig. 3).

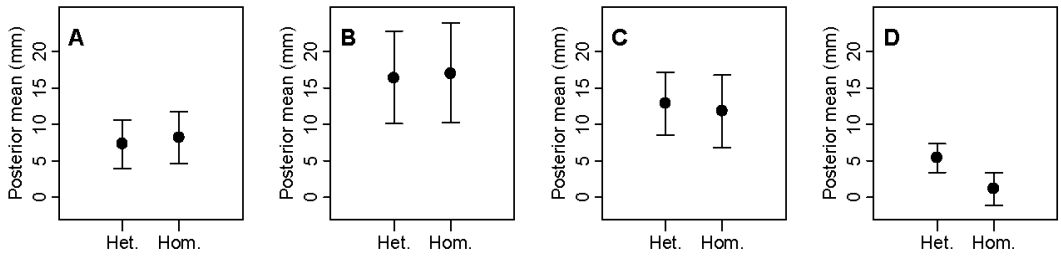

**Table 1.** Models of quantitative-trait evolution relevant to this study with their parameters and biological interpretation, indicating for each model whether the optimal trait value, $\theta$, the intensity of random fluctuations in the evolutionary trajectory, $\sigma^2$, and the selective "pull" toward the optimal value, $\alpha$, are modeled with one global parameter or with two parameters that are heterostyly- and homostyly-specific.

| Model | Parameters | | | Interpretation for quantitative trait evolution |
|---|---|---|---|---|
| | *Theta* | *Sigma²* | *Alpha* | |
| $BM_1$ | Global | Global | - | Evolution is random and not affected by the loss of heterostyly. |
| $BM_S$ | Global | State-specific | - | Evolution is random and the loss of heterostyly changes the stochasticity of the floral trait. |
| $OU_1$ | Global | Global | Global | Evolution is directed toward an optimum value without being affected by the loss of heterostyly. |
| $OU_M$ | State-specific | Global | Global | The loss of heterostyly is associated with a shift toward a different optimal value. |
| $OU_{MA}$ | State-specific | Global | State-specific | The loss of heterostyly is associated with a shift toward a different optimal value that exerts a different selective pull. |
| $OU_{MV}$ | State-specific | State-specific | Global | The loss of heterostyly is associated with a shift toward a different optimal value and a change in stochasticity. |

Note that the information in the data was insufficient to fit $OU_{MVA}$ models (containing heterostyly- and homostyly-specific $\theta$, $\alpha$ and $\sigma^2$), hence, these were not further considered (see text).

**Table 2.** Model fit and estimated parameters of supported (AICc weight > 0.05) OUwie models.

| Trait | Model | AICc | AICc weight | *Theta* | | *Sigma*$^2$ | | *Alpha* | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Homostyly-specific | Heterostyly-specific | Homostyly-specific | Heterostyly-specific | Homostyly-specific | Heterostyly-specific |
| Calyx length | $OU_1$ | 17.91 ± 0.514 | 0.501 ± 0.008 | *0.682 ± 0.001* | | *0.068 ± 0.002* | | *0.434 ± 0.015* | |
| | $OU_M$ | 19.051 ± 0.516 | 0.284 ± 0.004 | 0.532 ± 0.005 | 0.690 ± 0.001 | *0.082 ± 0.006* | | *0.546 ± 0.039* | |
| | $OU_{MA}$ | 20.909 ± 0.51 | 0.108 ± 0.003 | 0.531 ± 0.006 | 0.690 ± 0.001 | *0.081 ± 0.006* | | 0.534 ± 0.037 | 0.534 ± 0.038 |
| | $OU_{MV}$ | 20.922 ± 0.512 | 0.108 ± 0.003 | 0.529 ± 0.005 | 0.690 ± 0.001 | 0.081 ± 0.005 | 0.081 ± 0.006 | *0.535 ± 0.038* | |
| Corolla-tube length | $OU_{MA}$ | 198.623 ± 0.67 | 0.43 ± 0.009 | 1.281 ± 0.006 | 1.516 ± 0.002 | *0.325 ± 0.021* | | 0.629 ± 0.038 | 0.587 ± 0.038 |
| | $OU_{MV}$ | 198.281 ± 0.725 | 0.519 ± 0.01 | 1.290 ± 0.009 | 1.513 ± 0.002 | 1.191 ± 0.072 | 0.357 ± 0.027 | *0.649 ± 0.05* | |
| Corolla-limb diameter | $OU_1$ | 169.324 ± 0.263 | 0.428 ± 0.011 | *1.265 ± 0.001* | | *4.155 ± 0.348* | | *9.388 ± 0.786* | |
| | $OU_M$ | 171.099 ± 0.32 | 0.161 ± 0.003 | 1.208 ± 0.009 | 1.271 ± 0.001 | *3.927 ± 0.369* | | *8.871 ± 0.833* | |
| | $OU_{MV}$ | 169.314 ± 0.368 | 0.412 ± 0.013 | 1.202 ± 0.008 | 1.272 ± 0.001 | 7.730 ± 0.638 | 3.814 ± 0.315 | *9.749 ± 0.807* | |
| Herkogamy | $OU_{MA}$ | -62.412 ± 0.492 | 0.067 ± 0.012 | 0.045 ± 0.003 | 0.528 ± 0 | *0.173 ± 0.008* | | 2.183 ± 0.095 | 2.225 ± 0.096 |
| | $OU_{MV}$ | -68.812 ± 0.65 | 0.917 ± 0.014 | 0.066 ± 0.002 | 0.531 ± 0 | 0.034 ± 0.008 | 0.228 ± 0.022 | *2.860 ± 0.275* | |

Parameter estimates are reported as mean ± standard error across 100 stochastic maps generated using transition model 3 for the evolution of heterostyly. See Table S1 in the Supporting information for full results including models with AICc weight <0.05. for the four floral traits, indicating corrected AIC score (AICc), AICc weight, and the estimated values of the parameters $\theta$ (theta; optimum in cm), $\alpha$ (alpha, selective pull) and $\sigma^2$ (sigma$^2$; rate of random drift). When models contain a single, global parameter, estimates are italicized and printed in the center of the column; estimates for heterostyly- and homostyly-specific parameters are reported in their respective columns. Note that in models with heterostyly- and homostyly-specific parameters, the inferred optima, $\theta$, is lower in homostylous species in nearly all models, whereas the rate of macroevolutionary drift, $\sigma^2$, is higher in homostylous species for all traits except herkogamy.